



Gary Ernest Smith. *Old Road, New Road*. Oil on canvas, 40" × 60". Courtesy of Raymond E. Johnson's Overland Gallery of Fine Art, Scottsdale, Arizona.

*Overexpression of ERCC1
may play a role in
cisplatin resistance in
non-small-cell lung cancer.*

Nucleotide Excision Repair Pathways Involved in Cisplatin Resistance in Non-Small-Cell Lung Cancer

*Rafael Rosell, MD, Miquel Taron, PhD, Agusti Barnadas, MD,
Giorgio Scagliotti, MD, Carme Sarries, PhD, and Barbara Roig, PhD*

Background: *In spite of the growing list of genetic abnormalities identified as being involved in DNA repair pathways that alter chemosensitivity in non-small-cell lung cancer (NSCLC) patients, translational assays have not yet been developed for use in individualized chemotherapy.*

Methods: *In metastatic NSCLC, no single cisplatin-based chemotherapy regimen has been shown to be superior to any other. Although these studies show a small survival tail at 3 years, the majority of patients had a median survival of 8 to 10 months. We review the principal mechanisms of cisplatin resistance, particularly those involved in the nucleotide excision repair (NER) pathways (transcription-coupled repair and global genomic repair).*

Results: *ERCC1 is a single-stranded DNA endonuclease that forms a tight heterodimer with xeroderma pigmentosum complementation group F. It incises DNA on the 5' side of a lesion such as cisplatin-DNA adduct. Therefore, overexpression of ERCC1 and other NER enzymes during ovarian cancer chemotherapy with cisplatin appears to be implicated in the formation of cellular and clinical drug resistance. Recently, baseline ERCC1 mRNA overexpression has been related to poor response and survival in cisplatin-treated NSCLC patients.*

Conclusions: *The level of evidence for many assays is limited, and only ERCC1 mRNA levels have been analyzed extensively. The impact of ERCC1 should be fully validated in prospective clinical trials.*

From the Medical Oncology Service, Hospital Germans Trias i Pujol, Badalona (Barcelona), Spain (RR, MT, AB, CS, BR), and the Thoracic Oncology Unit, Department of Clinical and Biological Sciences, University of Torino, Torino, Italy (GS).

Submitted August 5, 2002; accepted September 30, 2002.

Address reprint requests to Rafael Rosell, MD, Medical Oncology

Service, Hospital Germans Trias i Pujol, Ctra Canyet, s/n, 08916 Badalona (Barcelona), Spain. E-mail: rrosell@ns.bugtip.scs.es

Dr Scagliotti receives honoraria from Eli Lilly and Co, Aventis Pharmaceuticals Inc, and AstraZeneca Pharmaceuticals LP. The other authors report no significant relationship with the companies/organizations whose products or services are referenced in this article.

Introduction

Cisplatin has long been the foundation of chemotherapy in lung cancer, and the role of noncisplatin combinations has not yet been fully demonstrated. Cisplatin is the platinum agent of choice in the treatment of germ-cell tumors. On the other hand, oxaliplatin is effective in colorectal cancer, unlike cisplatin, and shows higher activity in vitro in cancer cell lines with an impaired DNA mismatch repair (MMR) system.¹ The mechanisms of resistance to cisplatin have recently been reviewed in depth, illustrating how multiple proteins and several pathways intervene in this resistance.¹

Although cisplatin or carboplatin are the essential partners in combination chemotherapy in non-small-cell lung cancer (NSCLC), there are still many paradoxical findings. In a European study, more than 400 patients with stage IIIB (30%) or IV (70%) NSCLC were randomized to receive cisplatin 100 mg/m² or a combination of paclitaxel 175 mg/m² by 3-hour infusion plus cisplatin 80 mg/m² every 3 weeks. Although differences in response were observed in favor of the combination, there were no differences in median survival (8.6 months in the cisplatin arm and 8.1 months in the combination arm).² These results are open to various explanations, ranging from the low paclitaxel dose to the differences in the cisplatin dose between the two arms. Similarly, the Cancer and Leukemia Group B (CALGB) has reported its trial of paclitaxel 250 mg/m² by 3-hour infusion plus carboplatin at a dose based on the area under the concentration-time curve (AUC) of 6 compared with paclitaxel alone at the same dose. The same phenomena were observed: significant differences in response and median survival³ in favor of the combination regimen but similar 1-year survival in the two arms.⁴

In the landmark four-arm Eastern Cooperative Oncology Group (ECOG) trial in advanced NSCLC, all four platinum-based combination chemotherapy regimens attained the same pattern of response, median survival, and 1- and 2-year survival rates. In the control arm (cisplatin 75 mg/m² plus paclitaxel 135 mg/m² by 24-hour infusion), the response rate was 21%, median survival was 7.8 months, 1-year survival was 31%, and 2-year survival was 10%. Similar results were observed in patients treated with cisplatin plus gemcitabine, cisplatin plus docetaxel, and carboplatin plus paclitaxel⁵ (Table 1).

In the three-arm Italian Lung Cancer Project trial, patients were randomized to receive gemcitabine plus cisplatin or paclitaxel plus carboplatin or vinorelbine plus cisplatin. Again, no differences in response rate (30%), time to progression (4.6 months), or median survival (9.8 months) were observed.⁶ However, in the first trial comparing cisplatin plus paclitaxel vs carboplatin plus paclitaxel, differences in time to progression (4.2 vs 3 months; $P=.03$) and median survival (9.8 vs 8.2 months; $P=.01$) were observed in favor of the cisplatin arm⁷ (Table 1).

These results can be interpreted as a reflection of the failure of chemotherapy in advanced NSCLC, which seems unable to progress beyond the frontier of 8-month median survival. However, the ECOG trial⁵ has ushered in a new era in cancer management that will include not only the concepts of new therapeutic targets, chemoprevention, and spiral computed tomography screening,⁸ but also the genetic bases of chemoresistance, which can pave the way for tailored chemotherapy. Cisplatin damages DNA, inducing the formation of chemically stable DNA adducts. The absence of measurable cisplatin DNA adducts, determined by enzyme-linked immunosorbent assay (ELISA),

Table 1. — Outcomes in Selected Non-Small-Cell Lung Cancer Trials

	Response Rate	Survival		Median Time to Progression (mos)
		Median (mos)	1 Year	
Schiller et al ⁵				
Pac/cis	21%	7.8	31%	3.4*
Gem/cis	22%	8.1	36%	4.2*
Docetaxel/cis	17%	7.4	31%	3.7
Pac/carbo	17%	8.1	34%	3.1
Scagliotti et al ⁶				
Vrb/cis	30%	9.5	37%	4.6
Gem/cis	30%	9.8	37%	5.3
Pac/carbo	32%	10.0	43%	5.5
Pac/cis = paclitaxel/cisplatin				
Gem/cis = gemcitabine/cisplatin				
Pac/carbo = paclitaxel/carboplatin				
Vrb/cis = vinorelbine/cisplatin				
* Significant difference in time to progression between the paclitaxel/cisplatin control arm and the gemcitabine/cisplatin arm ($P=.001$).				

is associated with poor outcome. In one study, multiple tissues, including ovarian tumor, were obtained at autopsy from 8 patients who had received either cisplatin or carboplatin chemotherapy. Cisplatin DNA adducts were detected in most of the tissues examined, and DNA adduct levels were similar in the majority of tissues from the same subject, whether taken from the bone marrow, liver, brain, or peripheral nerve.⁹ The assessment of DNA adduct levels could become one predictive assay for cisplatin and/or radiotherapy. Schaake-Koning et al¹⁰ observed an improvement in survival in patients with locally advanced NSCLC who were treated with daily radiotherapy plus daily cisplatin 6 mg/m² approximately 1 hour before irradiation (20 administrations for a total of 120 mg/m²), compared with radiotherapy alone. Three-year survival was 16% for concomitant cisplatin-radiotherapy vs 2% for radiotherapy alone.

Differences in survival according to cisplatin DNA adduct levels have been observed in patients treated with concomitant cisplatin and radiotherapy. Dutch investigators studied 27 patients treated with daily cisplatin and radiotherapy.¹¹ To assess cisplatin DNA adduct levels, buccal cells were collected by wiping the inner cheek with a cotton swab before cisplatin treatment and 1 hour after the fifth course of cisplatin. The immunohistochemical method used for cytosins enabled cisplatin DNA adducts to be visualized in nuclei of the buccal cells. Nuclear signal intensity (arbitrary units) ranged from 0.4 to 2.8. Striking differences in survival were observed according to DNA adduct levels. Thirteen patients with low DNA adduct levels (≤ 1.16) had a median survival of 5.2 months compared to 14 patients with high levels (> 1.16), who had a median survival of 30.2 months ($P < .0001$). If these data are validated in a large-scale study, cisplatin DNA adduct levels could be used to identify the nearly 50% of patients who could obtain the greatest benefit from a concomitant cisplatin-radiotherapy approach and enable us to look for a different therapy for the 50% who would not benefit from such an approach.

Several molecular assays can be used to tailor chemotherapy in the care of lung cancer patients. Accumulated evidence indicates that several genetic markers are related to cisplatin resistance, and current research is providing hints that predictive markers may also affect resistance to gemcitabine and/or microtubule-interacting drugs.

DNA Repair

Cell repair capacity is stored in the linear sequence of approximately 3×10^9 copies of the four bases — guanine, cytosine, adenine and thymine — aligned in

the DNA. A growing number of reports identify DNA damage with the regulation of DNA repair gene transcription and the control of cell cycle progression and apoptosis via DNA damage checkpoints. Different pathways of DNA repair are polymorphic and vary interindividually and with age. These features influence the chemosensitivity of tumor cells toward DNA-reactive cytotoxic drugs.

DNA repair is a counteragent in carcinogenesis and an accomplice in cancer therapy resistance.¹² There are several major DNA repair pathways. Excision repair, including nucleotide excision repair (NER), has been strongly linked to cisplatin resistance. Base excision repair (BER) also plays an important role in chemotherapy resistance. The mRNA levels of the excision repair cross-complementing (*ERCC1*) gene, involved in the NER pathway, have recently been found to be closely correlated with levels of 8-oxoguanine DNA glycosylase (*OGG1*), involved in the BER pathway. The repair of double-strand breaks, induced by cytotoxic agents, radiotherapy, and reactive oxygen species, is carried out by homologous recombination and nonhomologous DNA end joining. Other pathways are MMR and one-step repair (OSR), meaning the direct reversal of DNA damage. The repair protein O⁶-alkylguanine-DNA alkyltransferase, also known as O⁶-methylguanine-DNA methyltransferase (MGMT), intervenes in OSR through removal of an alkyl group from the O⁶-atom of guanine in the DNA of cells exposed to alkylating agents. With increasing size of the alkyl group, the relative contribution of MGMT to the repair of O⁶-alkylguanines in DNA decreases and excision repair becomes more relevant.¹² As an example of OSR, treatment with chloroethylnitrosourea (BCNU) correlates with MGMT activity; in the process of cytotoxic interstrand cross-links in target cell DNA, BCNU initially alkylates the O⁶-atom of guanine. Intriguingly, MGMT levels vary greatly among tumors, which has been used in pharmacogenomic interpretation. Hypermethylation of MGMT (abrogating OSR) was observed in 40% of brain tumors treated with BCNU and was related to significantly better survival.¹³ Interestingly, the activity of temozolomide has been linked to tumor MGMT. However, when temozolomide was combined with CPT-11, this mechanism of resistance was circumvented in tumor cells that were either MGMT proficient or MMR deficient.¹⁴

The possibility of individualizing DNA repair profiles is becoming a central issue in the search for improved chemotherapy results. Current bioinformatics tools for microarray data have correlated gene expression profiles in cell lines with response to chemotherapy, leading to the identification of genes that may be important for drug sensitivities. However, profiling with microarray requires relatively large

quantities of RNA, making the process inappropriate for certain applications. Cancer cells accumulate multiple genetic abnormalities in signal transduction pathways during carcinogenesis and cancer progression. NER deficiencies are related to lung oncogenesis yet simultaneously confer a chemotherapy advantage. Like many DNA alkylators, cisplatin acts as a cross-linker, inhibiting DNA replication, which is the critical target in cancer chemotherapy. Cross-links between guanine bases are induced by cisplatin, carboplatin, and oxaliplatin. Cisplatin and carboplatin form an identical cross-link, while the oxaliplatin cross-link is structurally different due to the bulky 1,2-diaminocyclohexane group in the adduct.¹⁵ The mechanisms of DNA repair have been investigated in depth, primarily the nuclear DNA repair pathways involving BER, recombination, MMR, NER, and OSR. Mitochondrial DNA repair pathways can also play an important role and are reviewed elsewhere.¹⁶ In this review, we focus primarily on the NER pathway.

DNA Repair Capacity, Lung Cancer Risk, and Chemoresistance

Many cancer chemotherapeutic agents, including cisplatin, cause interstrand cross-links, which accounts for their therapeutic cytotoxic properties. Similarly, many carcinogens are bifunctional, causing both monoadducts and intrastrand or interstrand cross-links in DNA. DNA repair capacity (DRC) is genetically determined; it modulates lung cancer susceptibility and treatment response.¹⁷ Carcinogen-induced DNA damage induces breaks in the sugar-phosphate DNA backbone, either in one or both of the two strands of the double helix. Covalent binding of the carcinogen results in the formation of a chemically altered base in DNA that is called an adduct. A nucleotide adduct is a fragment consisting of carcinogen-base-deoxyribose-phosphate, a nucleoside adduct consists of carcinogen-base-deoxyribose, and a base adduct is the carcinogen-modified base only. DRC has been assessed in peripheral blood lymphocytes by the host-cell reactivation assay, which measured cellular reactivation of a reporter gene damaged by exposure to 75 μm benzo[*a*]pyrene diol epoxide (BPDE).¹⁸ With this functional assay, the mean level of DRC was significantly lower in patients with lung cancer than in controls. Younger cases (<65 years of age) and smokers were more likely than controls to have reduced DRC.¹⁸ BPDE-induced DNA adducts are repaired by the NER pathway, in which *ERCC1* plays a pivotal role, raising the hypothesis that lung cancer patients with lower *ERCC1* levels — and thus lower DRC — may have enhanced response and survival with cisplatin-based chemotherapy.

In an experimental model, elevated DRC was associated with resistance to cisplatin in lung cancer cell lines.¹⁹ In this case, the overall DRC was estimated based on the ability of cells to reactivate the pRSV-CAT (chloramphenicol acetyltransferase) plasmid damaged by cisplatin. The pRSV-CAT plasmid contains the bacterial gene for CAT under the control of the RSV long-terminal repeat promoter. Platination of the pRSV-CAT plasmid will diminish or abolish CAT gene expression as a consequence of DNA damage after transfection into cells. Repair of these lesions will restore CAT gene expression and provide information about the overall repair capacity of a given cell population. NSCLC cells were found to be significantly more resistant to cisplatin than small-cell cancer cell lines isolated from untreated patients.¹⁹

The epidemiology of DRC and its effect on cancer susceptibility has been fully developed. In 1998, 64 reports addressed the association of cancer susceptibility with defects in DRC.²⁰ Several assays of DRC have been used. With the host-cell reactivation assay, DRC has been measured in peripheral blood lymphocytes with the host-cell reactivation assay and calculated as the percentage of residual CAT gene expression after the repair of ultraviolet radiation- or cisplatin-damaged plasmid DNA divided by that in undamaged plasmid DNA. The host-cell reactivation assay measuring the activity of the CAT gene has been used in cells transfected with BPDE-treated plasmid. Because a single unrepaired DNA adduct can effectively block CAT transcription, any CAT activity will reflect the ability of the transfected cells to remove BPDE-induced adducts from the plasmids. The most susceptible subgroup of cigarette smokers on the basis of their low DRC were case patients who were young (<60 years if age), female, or light smokers, or who reported a family history of cancer. In contrast, in a study comparing 316 newly diagnosed lung cancer patients and 316 cancer-free control subjects, heavy smokers among both case patients and control subjects tended to have more proficient DRC than lighter smokers, suggesting that cigarette smoking may stimulate DRC in response to the DNA damage caused by tobacco carcinogens.²⁰ Similarly, women smokers with the GSTM1 null genotype, which results in diminished glutathione S-transferase (GST) activity, had the greatest lung cancer risk compared with other groups of women and men with different GSTM1 genotypes. The absence of detoxifying GST activity may result in an excess of internal exposure to tobacco carcinogens, leading to a higher level of DNA damage or adduct formation.²¹

In short, defective DRC is one of the major factors responsible for carcinogenesis, and at the same time, it can confer a favorable cytotoxic effect. Preliminary hints as to the therapeutic benefit of deficient DRC

stem from molecular epidemiology studies assessing the DRC by the host-cell reactivation assay in lymphocytes, which measures the NER capacity.²² As stated above, one determinant of the level of cisplatin DNA adducts in host tissues (cancer patients) treated with cisplatin-based chemotherapy is the rate of DNA repair. Subjects vary considerably in their capacity to remove DNA adducts.¹⁸ It is thought that NER is the primary mechanism for repairing cisplatin DNA adducts. The relationship between DRC and survival in patients with NSCLC treated with cisplatin-based chemotherapy was recently examined.²² In this study, patients who had received chemotherapy were divided into quartiles according to their DRC. Patients in the top quartile (DRC >9.2%) had a risk of death that was more than two times the risk of death for patients in the bottom quartile (DRC <5.8%; $P=0.01$). Median survival was 8.9 months for patients in the top quartile compared with 15.8 months for those in the bottom quartile ($P=0.04$). Intriguingly, among the 36 chemo-naïve patients who underwent curative surgical resection, there was a slight survival advantage associated with increased DRC. This finding could be relevant when interpreting the results of neoadjuvant chemotherapy trials in early NSCLC. The assessment of DRC, either by measuring cisplatin DNA adducts, the host-cell reactivation assay, or the overexpression of *ERCC1* gene transcript, is warranted in such trials to identify the subgroup of patients with low DRC, who could have lower survival when treated with surgery alone and at the same time could benefit from neoadjuvant or adjuvant chemotherapy. In contrast, patients with high DRC could have better survival when treated with surgery alone and could be refractory to neoadjuvant or adjuvant approaches.

NER Capacity and Cisplatin Effect

It is a common belief that cisplatin exerts its cytotoxic effect by disrupting the DNA macromolecule, mainly through the formation of intrastrand adducts and interstrand cross-links that are repaired through the NER pathway. It is also postulated that tumors that are defective in MMR become more resistant to cisplatin than their MMR-proficient counterparts. The NER pathway consists of several steps: damage recognition, dual incision/excision, repair synthesis, and ligation. Approximately 30 proteins participate in this repair process; above all, *ERCC1* has a crucial role in the incision process, which is the rate-limiting step of the pathway. *ERCC1* is a 15-kb repair gene located on human chromosome 19. *ERCC1* forms a heterodimer with *XPF*, and the *ERCC1/XPF* complex is responsible for the incision to cleave the damaged strand at the phosphodiester bonds between 22 and 24 nucleotides 5' to the lesion.

Functional *ERCC1* is important in the repair of cisplatin DNA adducts and in cisplatin sensitivity in intact cells.²³ *ERCC1* mRNA levels, measured by quantitative polymerase chain reaction (PCR), were examined in gastric cancer patients treated with cisplatin/fluorouracil (FU). Before chemotherapy, cDNA was obtained from primary gastric tumors, and *ERCC1* mRNA levels were expressed as the ratio of the PCR product of the *ERCC1* gene and the β -actin housekeeping gene. The median *ERCC1* mRNA level for the 17 responders was 4.9, while the median *ERCC1* mRNA level for the 16 resistant patients was 8. The difference between responders and nonresponders was statistically significant.²³ The median survival for patients with *ERCC1* mRNA levels <5.8 was not reached at the time the report was published, while the median survival for those with levels >5.8 was only 5.4 months. The difference was highly significant, disclosing for the first time that intratumoral levels of *ERCC1* mRNA influence the outcome of gastric cancer patients treated with cisplatin/FU.²³ This study gave no conclusive results on whether *ERCC1* mRNA levels could be an independent predictive marker for cisplatin benefit. Originally, *ERCC1* mRNA levels were assessed in ovarian cancer tissue harvested from 28 patients before treatment with carboplatin- or cisplatin-based chemotherapy. RT-PCR-based assay was used to determine the level of expression of *ERCC1* and β -actin, as well as human xeroderma pigmentosum group A correcting (*XPAC*) gene. Numerical values for the expression of the *ERCC1* and *XPAC* genes in the ovarian tumor tissue samples were obtained using the densitometric readout of the autoradiographic signal generated by the 32 P-labeled *ERCC1* or *XPAC* probe divided by the densitometric reading for β -actin. In this case, the numerical values were different from those reported in the literature using quantitative PCR. Thirteen nonresponders showed greater levels of *ERCC1* mRNA than 15 responders.²⁴

The cisplatin effect on *ERCC1* mRNA expression has been examined in vitro. In response to a 1-hour cisplatin exposure, human ovarian cancer cells showed a two- to six-fold increase in steady-state levels of *ERCC1* mRNA over basal expression levels.^{25,26} A positive association has also been demonstrated between the level of *ERCC1* mRNA expression and the amount of cisplatin-DNA adduct repair in ovarian tumor cells in vitro.²⁶

Intriguingly, *ERCC1* antisense RNA abrogates the gemcitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells that were proficient in NER. Experimental results indicate that stable expression of *ERCC1* antisense mRNA downregulates the level of mRNA and repair activity. The downregula-

tion of the repair activity significantly correlates with the reduction of the cytotoxic synergism between gemcitabine and cisplatin.²⁷

Along the same lines, *ERCC1* mRNA levels have been correlated with oxaliplatin resistance in colorectal cancer patients. Median survival for patients with *ERCC1* expression <4.9 was 10 months, while for patients with *ERCC1* expression >4.9, it was 1.9 months.²⁸ These findings indicate that intratumoral *ERCC1* mRNA may be an independent predictive marker for oxaliplatin combination chemotherapy. Both this study and the original study in gastric cancer²³ were conducted by investigators from the University of Southern California (USC)/Norris Comprehensive Cancer Center and Response Genetics. *ERCC1* mRNA levels have not been correlated with noncisplatin chemotherapy response. When the correlation between the expression of eight genes involved in NER and DRC was examined, only *ERCC1* and *XPD* mRNA levels were highly correlated both with each other and with DRC.²⁹ *ERCC1* and *XPD* (also known as *ERCC2*) are closely linked on chromosome 19q13.2-13.3. More recently, the same investigators observed a strong correlation between *ERCC1* and *OGG1* mRNA levels in peripheral lymphocytes. *OGG1* encodes the 8-oxoguanine-DNA glycosylase, which removes 8-oxoguanine from DNA as part of the BER pathway.³⁰ *XPD* polymorphism has been related to lower DRC.³¹ Approximately half of the population examined had the genotype Lys751Lys and also had Asp312Asp. These patients had a good DRC and therefore are presumably resistant to cisplatin. In an epidemiological study matching 341 lung cancer cases with 360 smoker control subjects, a host-cell reactivation assay measuring the activity of the CAT gene was used in cells transfected with plasmids treated with BPDE. DRC was lower in the lung cancer patients than in the controls. The variants Gln751Gln and Asn312Asn had suboptimal DRC, with a significant increase in the hazard ratio, in contrast with the wild-type genotypes both in cases and controls, which exhibited the most proficient DRC.³¹ The frequency of homozygous variants was 10% for either codon. In an intermediate group with heterozygous polymorphisms, the frequency was 40% at either codon.

The clinical interest of these findings lies in their potential usefulness in identifying in constitutional DNA from lymphocytes the polymorphisms associated with suboptimal DRC and their potential role in identifying patients with better response to cisplatin chemotherapy.

UV light, BPDE DNA adducts, and cisplatin DNA adducts are effective blocks to RNA polymerase II and thus block transcription. These DNA lesions are

removed by NER, which is split into subpathways: transcription-coupled repair (TCR) and global genomic repair (GGR). Importantly, TCR repairs transcription-blocking lesions in transcribed DNA strands of active genes, whereas GGR repairs the lesions in the nontranscribed strand of active genes and nontranscribing genome.³² When transcribing RNA polymerase II encounters the lesion, two TCR-specific factors, CSA and CSB, are implicated for the activation of the common NER molecular pathway.³² The clinical implications of TCR lie in the fact that cisplatin-resistant tumors show an intact TCR system, while tumors are sensitive to cisplatin when the TCR subpathway is deficient. For GGR, the xeroderma pigmentosum group C (*XPC*) complex is activated. Along with the basal transcription factor (TFIIH), an *XPG* binds to the DNA around the lesion. TFIIH contains two helicases, *XPB* and *XPD*, which open approximately a 30-base-long DNA segment around the damage. This open intermediate is stabilized by replication protein A and *XPA*. The DNA strand that contains the damaged base(s) is excised by the two NER endonucleases, *XPG* and *XPF/ERCC1*. *XPG* cleaves the damaged DNA strand 3' from the lesion, and *XPF/ERCC1* cleaves the damaged strand 5' from the DNA lesion. Finally, the resulting gap is filled in by DNA polymerases in the presence of replication factors. Molecular deficiencies (in both GGR and TCR subpathways) in primary fibroblasts confer marked hypersensitivity to cisplatin compared to normal primary fibroblasts. These results demonstrate that any one deficiency in *XPA*, *XPD*, *XPF*, or *XPG* confers marked hypersensitivity to cisplatin.³²

ERCC1 Expression

We have analyzed the role of *ERCC1* expression in metastatic NSCLC patients treated with gemcitabine/cisplatin. mRNA was isolated from paraffin-embedded primary tumor specimens obtained by bronchoscopy biopsy. The median *ERCC1* expression in 56 patients analyzed relative to the expression of the control β -actin was 6.7. Patients with *ERCC1* expression above 6.7 had a median survival of 5 months, while those with lower levels had a median survival of 15 months. This difference was statistically significant, and importantly, in a Cox multivariable analysis, *ERCC1* levels surfaced as an independent predictive variable. The fact that the cutoff was higher than previously described indicates that a certain level of *ERCC1* is required for synergism between gemcitabine and cisplatin.³³

The potential role of the 5'-untranslated region (5'-UTR) in *ERCC1* mRNA expression has also been examined. RT-PCR was carried out with primers targeting the 5'-UTR to amplify a fragment containing exon I

(UTR) and exon II (containing the initiation codon) of the *ERCC1* gene in 121 ovarian cancer samples. Interestingly, two PCR amplimers from the same sample for the target segment within the UTR appeared in some samples. The prevalence of the two amplimers occurred in the group of patients with high *ERCC1* mRNA levels (48%). In contrast, only 5% of patients with a single amplimer showed high *ERCC1* mRNA levels. Direct DNA sequencing of the cDNA from each of the 121 ovarian cancer tumor samples confirmed that tumors with two amplimers contained two distinct sequences. The longer sequences included the complete target sequence, 261-bp (wild type), and the shorter sequences demonstrated a 42-bp deletion.³⁴ This 42-bp deletion seems to be associated with high *ERCC1* mRNA levels.

Overall, *ERCC1* stands out as a potential predictive marker for cisplatin-based chemotherapy and it could be the basis for customized chemotherapy.

Other Genetic Markers Conferring Cisplatin Resistance

Defects in DNA MMR may result from mutation or methylation-mediated silencing of three mismatch repair genes: hMLH-1, hMSH-2, or hPMS-2. These defects have been shown to be a mechanism of resistance to cisplatin but not to oxaliplatin both in vivo and in vitro.³⁵ It is thought that an MMR complex recognizes cisplatin-DNA adducts but not oxaliplatin-DNA adducts, and that MMR proteins are involved in mediated response to DNA damage.^{35,36} This has been demonstrated in experiments using MMR-proficient and MMR-deficient cells, where different DNA repair pathways have been linked to cisplatin but not to oxaliplatin.^{37,38}

In addition to members of the MMR family, other proteins also interact with cisplatin DNA adducts, including numerous nuclear proteins binding to cisplatin DNA adducts, such as linker histones H1, high mobility group 1 (HMG1) box-containing proteins, and many different transcription factors. HMG1 is a nonhistone chromosomal protein that appears to be involved in DNA replication and repair. HMG1 was overexpressed in three cisplatin-resistant cell lines. The expression of HMG1 is increased at the transcriptional level, which may be due to enhanced activity of the CCAAT-binding transcription factor/nuclear factor 1 (CTF/NF-1).³⁹

Other mechanisms of cisplatin resistance involve decreased net intracellular accumulation of cisplatin. ATP-dependent pathways activate outward efflux of cisplatin through the plasma membrane, which is

enhanced in some cisplatin-resistant cell lines.⁴⁰ Multidrug resistance protein 2 (MRP2) is also a putative cisplatin efflux pump.⁴¹ Other mechanisms involve cisplatin detoxification by glutathione/glutathione acetyl-S-transferases (GSH/GST). The four major GST-related genes (GSTP1, GSTT1, GSTM1, and GSTZ1) attach reduced glutathione to electrophilic groups in a wide variety of toxic compounds, including chemotherapeutic agents, and GSTP1 overexpression has been correlated with increased cisplatin resistance.⁴² Recently, the GSTP1 Ile105 Val polymorphism has been associated with increased survival in patients with advanced colorectal cancer receiving FU/oxaliplatin chemotherapy.⁴³ This polymorphism has been related to substantially diminished GSTP1 enzyme activity, reducing the detoxification pathway mediated by GSTP1.⁴⁴

Metallothioneins are also involved in low cisplatin-adduct formation. Metallothioneins are metal binding proteins of low molecular weight; they are cysteine rich and have an important role in the homeostasis of trace metals and in the detoxification of metals such as Cd²⁺ and Hg²⁺. Metallothioneins are transcriptionally induced by these metals through metal-responsive elements located in the 5'-regulatory regions of human metallothionein genes. Overexpression of metallothionein has been correlated with cisplatin-resistance, and transfection of the gene encoding for metallothionein increases cisplatin resistance.⁴⁵

BRCA1 plays an important role in DNA damage repair mediated-cisplatin sensitivity.⁴⁶ Increased levels of BRCA1 have been observed in cisplatin-resistant breast and ovarian carcinoma cell lines derived from MCF7 and SKOV3. Furthermore, antisense inhibition of BRCA1 in SKOV3 cisplatin-resistant cell lines resulted in increased sensitivity to cisplatin, decreased DRC, and increased apoptosis. BRCA1 and Rad51¹⁶ co-localize and interact in the S-phase of the cell cycle.⁴⁷

Conclusions

Preclinical data indicate that TCR is involved in cisplatin resistance. However, clinical findings, with a level of evidence of 2 (positive phase II studies), have focused on *ERCC1*, which is involved in GGR. Further research is required to validate *ERCC1* mRNA levels in randomized trials, and customized chemotherapy trials including *ERCC1* assessment are ongoing. However, an important limitation is the availability of tumor samples. Stage IV NSCLC is often diagnosed through cytology, which can impede *ERCC1* assessment. In addition, in some instances, the amount of tumor tissue in bronchial biopsies is scarce. Another test to assess the GGR pathway is the host-cell reactivation assay, which

Table 2. — Predictive Tests Considered for Use in Cisplatin-Based Customized Chemotherapy Trials

Marker	Assay	Level of Evidence
<i>ERCC1</i>	cDNA quantitation	2
Damaged CAT reporter genes repaired in test lymphocytes transfected with BPDE-treated plasmids	Host-cell reactivation	2
Cisplatin-DNA adducts	Immunohistochemistry	2
Ap endonuclease	Immunohistochemistry	3
<i>XPD</i> polymorphisms	Genotyping	2/3

examines the ability to repair BPDE-induced DNA adducts in peripheral lymphocytes. Like the assessment of *ERCC1*, this test has a level of evidence of 2. A third test is the measurement of cisplatin-DNA adducts in nuclei of buccal cells, which also has a level of evidence of 2. The only limitation for this method is that it requires at least the prior administration of one cycle of cisplatin-based chemotherapy. Cisplatin-DNA adducts can be detected a few days after the first cycle, and the results can be used by the medical oncologist when deciding whether to continue with cisplatin or switch to a noncisplatin regimen in those cases with poor nuclear signal intensity (Table 2).

The role of adjuvant chemotherapy has not yet been demonstrated in NSCLC, and there are some hints that patients with proficient GGR can have better prognosis — though paradoxically, proficient GGR can cause resistance to cisplatin-based adjuvant chemotherapy. The assessment of *ERCC1* in surgical samples could be used to select patients with low *ERCC1* levels who could most benefit from adjuvant chemotherapy. Along the same lines, apurinic/aprimidinic endonuclease (Ap endo) is a key DNA repair enzyme that confers resistance to radiotherapy and alkylating drugs. Ap endo is crucial in the BER pathway. High nuclear Ap endo expression correlated with better survival in surgically resected NSCLC patients.⁴⁸ At the same time, it is reported to be a mechanism of resistance to alkylating agents.⁴⁹ Finally, *XPD* polymorphisms can be examined in constitutional DNA, and for this reason, lymphocytes from peripheral blood are a practical source of DNA. The level of evidence for this test is 2/3 (ie, little clinical evidence).

References

- Guminski AD, Harnett PR, deFazio A. Scientists and clinicians test their metal-back to the future with platinum compounds. *Lancet Oncol.* 2002;3:312-318.
- Gatzemeier U, von Pawel J, Gottfried M, et al. Phase III comparative study of high-dose cisplatin versus a combination of paclitaxel and cisplatin in patients with advanced non-small-cell lung cancer. *J Clin Oncol.* 2000;18:3390-3399.
- Lilenbaum RC, Herndon J, List M, et al. Single-agent (SA) versus combination chemotherapy (CC) in advanced non-small cell lung cancer (NSCLC): a CALGB randomized trial of efficacy, quality life (QOL), and cost-effectiveness. *Proc Annu Meet Am Soc Clin Oncol.* 2002;21:2. Abstract.
- Garfield D. Two drugs better than one for NSCLC. *Lancet Oncol.* 2002;3:389.
- Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med.* 2002;346:92-98.
- Scagliotti GV, De Marinis F, Rinaldi M, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non-small cell lung cancer. *J Clin Oncol.* 2002;20:4285-4291.
- Rosell R, Gatzemeier U, Betticher DC, et al. Phase III randomized trial comparing paclitaxel/carboplatin with paclitaxel/cisplatin in patients with advanced non-small cell lung cancer: a cooperative multinational trial. *Ann Oncol.* 2002;13:1539-1549.
- Carney DN. Lung cancer: time to move on from chemotherapy. *N Engl J Med.* 2002;346:126-128.
- Poirier MC, Reed E, Litterst CL, et al. Persistence of platinum-amine-DNA adducts in gonads and kidneys of rats and multiple tissues from cancer patients. *Cancer Res.* 1992;52:149-153.
- Schaake-Koning C, van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med.* 1992;326:524-530.
- van de Vaart PJ, Belderbos J, de Jong D, et al. DNA-adduct levels as a predictor of outcome for NSCLC patients receiving daily cisplatin and radiotherapy. *Int J Cancer.* 2000;89:160-166.
- Rajewsky MF, Müller R. DNA repair and the cell cycle as targets in cancer therapy. In: Alison M, ed. *The Cancer Handbook*. London: Nature Publishing Group; 2002:1507-1519.
- Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med.* 2000;343:1350-1354.
- Houghton PJ, Stewart CF, Cheshire PJ, et al. Antitumor activity of temozolomide combined with irinotecan is partly independent of O6-methylguanine-DNA methyltransferase and mismatch repair phenotypes in xenograft models. *Clin Cancer Res.* 2000;6:4110-4118.
- Siddik ZH. Mechanisms of action of cancer chemotherapeutic agents: DNA-interactive alkylating agents and antitumor platinum-based drugs. In: Alison M, ed. *The Cancer Handbook*. London: Nature Publishing Group; 2002:1295-1313.
- Kohn KW, Bohr VA. Genomic instability and DNA repair. In: Alison M, ed. *The Cancer Handbook*. London: Nature Publishing Group; 2002:87-106.
- Phillips DH. The formation of DNA adducts. In: Alison M, ed. *The Cancer Handbook*. London: Nature Publishing Group; 2002:293-307.
- Wei Q, Cheng L, Hong WK, et al. Reduced DNA repair capacity in lung cancer patients. *Cancer Res.* 1996;56:4103-4107.
- Zeng-Rong N, Paterson J, Alpert L, et al. Elevated DNA repair capacity is associated with intrinsic resistance of lung cancer to chemotherapy. *Cancer Res.* 1995;55:4760-4764.
- Wei Q, Cheng L, Amos CI, et al. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J Natl Cancer Inst.* 2000;92:1764-1772.
- Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J Natl Cancer Inst.* 2000;92:874-897.
- Bosken CH, Wei Q, Amos CI, et al. An analysis of DNA repair as a determinant of survival in patients with non-small-cell lung cancer. *J Natl Cancer Inst.* 2002;94:1091-1099.
- Metzger R, Leichman CG, Danenberg KD, et al. *ERCC1* mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol.* 1998;16:309-316.
- Dabholkar M, Vionnet J, Bostick-Bruton F, et al. Messenger RNA levels of *XPC* and *ERCC1* in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest.* 1994;94:703-708.
- Li Q, Gardner K, Zhang L, et al. Cisplatin induction of *ERCC-1* mRNA expression in A2780/CP70 human ovarian cancer cells. *J Biol Chem.* 1998;273:23419-23425.

26. Li Q, Yu JJ, Mu C, et al. Association between the level of ERCC-1 expression and the repair of cisplatin-induced DNA damage in human ovarian cancer cells. *Anticancer Res.* 2000;20:645-652.
27. Yang LY, Li L, Jiang H, et al. Expression of ERCC1 antisense RNA abrogates gemcitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells defective in mismatch repair but proficient in nucleotide excision repair. *Clin Cancer Res.* 2000;6:773-781.
28. Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol.* 2001;19:4298-4304.
29. Vogel U, Dybdahl M, Frenz G, et al. DNA repair capacity: inconsistency between effect of over-expression of five NER genes and the correlation to mRNA levels in primary lymphocytes. *Mutat Res.* 2000;461:197-210.
30. Vogel U, Moller P, Dragsted L, et al. Inter-individual variation, seasonal variation and close correlation of OGG1 and ERCC1 mRNA levels in full blood from healthy volunteers. *Carcinogenesis.* 2002;23:1505-1509.
31. Spitz MR, Wu X, Wang Y, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res.* 2001;61:1354-1357.
32. Furuta T, Ueda T, Aune G, et al. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. *Cancer Res.* 2002;62:4899-4902.
33. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res.* 2002;8:2286-2291.
34. Yu JJ, Thornton K, Guo Y, et al. An ERCC1 splicing variant involving the 5'-UTR of the mRNA may have a transcriptional modulatory function. *Oncogene.* 2001;20:7694-7698.
35. Fink D, Zheng H, Nebel S, et al. In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. *Cancer Res.* 1997;57:1841-1845.
36. Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. *Clin Cancer Res.* 1998;4:1-6.
37. Nehme A, Baskaran R, Nebel S, et al. Induction of JNK and cAbl signalling by cisplatin and oxaliplatin in mismatch repair-proficient and -deficient cells. *Br J Cancer.* 1999;79:1104-1110.
38. Vaisman A, Varchenko M, Umar A, et al. The role of hMLH1, hMSH3 and hMSH6 defects in cisplatin and oxaliplatin resistance: correlation with replicative bypass of platinum-DNA adducts. *Cancer Res.* 1998;58:3579-3585.
39. Nagatani G, Nomoto M, Takano H, et al. Transcriptional activation of the human HMG1 gene in cisplatin-resistant human cancer cells. *Cancer Res.* 2001;61:1592-1597.
40. Fujii R, Mutoh M, Sumizawa T, et al. Adenosine triphosphate-dependent transport of leukotriene C4 by membrane vesicles prepared from cisplatin-resistant human epidermoid carcinoma tumor cells. *J Natl Cancer Inst.* 1994;86:1781-1784.
41. Taniguchi K, Wada M, Kohno K, et al. A human canalicular multispecific organic anion transporter (cMOAT) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation. *Cancer Res.* 1996;56:4124-4129.
42. Ban N, Takahashi Y, Takayama T, et al. Transfection of glutathione S-transferase (GST)-pi antisense complementary DNA increases the sensitivity of a colon cancer cell line to adriamycin, cisplatin, melphalan, and etoposide. *Cancer Res.* 1996;56:3577-3582.
43. Stoehlmacher J, Park DJ, Zhang W, et al. Association between glutathione S-transferases P1, T1 and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst.* 2002;94:936-942.
44. Watson MA, Stewart RK, Smith GB, et al. Human glutathione S-transferase P1 polymorphisms. *Carcinogenesis.* 1998;19:275-280.
45. Vandier D, Calvez V, Massade L, et al. Transactivation of metallothionein promoter in cisplatin-resistant cancer cells: a specific gene therapy strategy. *J Natl Cancer Inst.* 2000;92:642-647.
46. Husain A, He G, Venkatraman ES, et al. BCRA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum (II). *Cancer Res.* 1998;58:1120-1123.
47. Scully R, Livingston DM. In search of the tumor-suppressor functions of BRCA1 and BRCA2. *Nature.* 2000;408:429-432.
48. Kakolyris S, Giatromanolaki A, Koukourakis M, et al. Nuclear localization of human AP endonuclease 1 (HAP1/Ref-1) associates with prognosis in early operable non-small-cell lung cancer (NSCLC). *J Pathol.* 1999;189:351-357.
49. Bobola MS, Blank A, Berger MS, et al. Apurinic/aprimidinic endonuclease activity is elevated in human adult gliomas. *Clin Cancer Res.* 2001;7:3510-3518.